35USC103(a)

The claims require at least six steps and the Action relies on Eberwine (US Pat No. 5,514,545) for teaching all but two of these (the converting and tagging steps) which it would remedy with Serafini (US Pat No. 6,114,152). In fact, Eberwine does teach the converting step, Serafini teaches all but one step, but neither Eberwine nor Serafini, alone or together, teach or suggest the normalization step. In fact, they teach away from it. But first, we respond directly to the Action.

Eberwine does not teach or suggest normalizing mRNA or aRNA as required by the claims, and does not teach or suggest normalizing cDNA vs. aRNA synthesis as relied upon by the Action at p.5, lines 9-10. At col.6, lines 1-3, Eberwine says that differences in efficiency of cDNA synthesis and aRNA amplification will serve to *normalize the analysis* within an experiment. The Action appears to misconstrue Eberwine's use of the word "normalize" and applies it out-of-context to cDNA and aRNA molecules. No where does Eberwine talk of normalizing DNA or RNA molecules as suggested. In fact, Eberwine is not talking about normalizing any "thing." What Eberwine says is that the expression profile of several different *mRNAs* within the *aRNA* population allows interexperimental comparisons of a given mRNA because differences in efficiency of cDNA synthesis will serve to *normalize the analysis*. Not the clearest language imaginable, but Eberwine does exemplify what he apparently means in the next two paragraphs.

In col.6, lines 4-40, Eberwine describes expression profiling of several hippocampal cells. In particular, Eberwine reports that he was able to separately classify hippocampal cells on the basis of the *ratio* of expression of specific mRNAs within the cell, wherein one class differed from others in the ratio of expression of K to Ca channel mRNAs. Eberwine cautions that this is not a quantitative measure of the amount of an individual mRNA, on a molar basis, but rather a 'self-controlled' comparison of hybridization intensities of these channels in the same aRNA population. In other words, the numbers of the ratio may not reflect molar amounts of the K and Ca channel mRNA. However, Eberwine assures, any *potential differences in amplification efficiency are normalized* by the similar autoradiographic intensities of other molecules used in the expression profiling, such as c-jun. In other words, Eberwine says that the differential

expression of genes, such as K or Ca channels, in different experiments can be compared because genes that do not change, such as c-jun, can be used to normalize the signal ratios of the K or Ca channels between experiments.

The Action also cites col.7, lines 30-35 of Eberwine. Here, not only is the cited portion of Eberwine taken out-of-context, it is placed in a horribly misleading context. The Action discusses Eberwine's use of RNA and cDNA and then says that Eberwine teaches that a vast excess of driver RNA is used to hybridize all available cDNA. By manufacturing this juxtaposition, the Action would have us believe that Eberwine suggests use of a normalization protocol in his methods. Nothing is further from the truth.

Eberwine's contribution is characterizing single cells based on their mRNA component. While acknowledging his method will not yield full-length clones, Eberwine argues at length that they should be of high complexity (beginning at col.5, line18). He describes two ways he assessed complexity of his aRNA population: expression profiling and cDNA library screening (col.5, lines 26-29) and reports his results with both (col.5, line 30 - col.6, line 40 and col.6, line 41 - col.7, line 28, respectively). Thereafter, Eberwine explains how complexity is historically measured (col.7, lines 29-37), why this traditional measure of complexity reflects not only distinctiveness, but also abundance (col.7, lines 37-48) and argues that it is easier to isolate low abundance mRNAs from a single cell (pursuant to his method) than from a tissue homogenate (prior methods)(col.7, lines 48-64). The portion of Eberwine cited by the Action is his description of how complexity is historically measured - Eberwine never does this and the whole point of his argument here is he doesn't need to – his method recovers low abundant mRNAs. Presumably, the Action's misleading implication was made through inadvertence.

No where does Eberwine suggest *normalizing molecules* - to the contrary, Eberwine says that any differential amplification is addressed by internal controls. Serafini does not remedy this defect as he does not teach normalizing RNA (the Action's statement to the contrary at p.7, line 5, is without support and inaccurate) or offer any motivation for modifying Eberwine to do so. Gudkov (US Pat No.5,866,327) does describe a conventional cDNA normalization protocol (e.g. col.11, lines 33-43) but does not suggests its use in the context of an amplification protocol such as claimed herein or as described by Serafini or Eberwine. Absent a prior art suggestion to

modify the protocol of Serafini (which describes the requisite tagging step not taught by Eberwine) to incorporate a normalization step as claimed, the claims are in compliance with 35USC103(a).

While the cited art does not suggest the claimed invention and does not support a prima facie case under 35USC103, for good measure, Applicants provide herewith an expert Declaration attesting to the foregoing and the fact that the cited art would not suggest the claimed invention to one of ordinary skill in the art. Note that the Declarants are three of the four coinventors of the cited Serafini et al. (the fourth coinventor, Serafini, is no longer at the University of California).

Because we recognize that the Examiner is under challenging time constraints and the subject matter of the invention is relatively arcane, we provide a brief overview of the field of the invention, largely derived from the background sections of Eberwine (see col.1-2) and the present disclosure (see p.1-3), to provide a context for appreciating the present invention.

The amount of mRNA in a single cell is too small for direct analysis. Prior to 1990, the way to address this problem was PCR amplification. However, PCR-based methods were found to bias toward producing short, extreme 3' fragments of mRNAs and with frequent introduction of erroneous bases (because of the high error rate of Taq polymerase). Furthermore, PCR would exponentially amplify these artifacts. The technique of amplified, antisense RNA (aRNA) circumvented these problems by introducing linear amplification with a relatively high fidelity polymerase. Eberwine extended aRNA technology with a second amplification step and applied the technique to single cells. Serafini also describes a double aRNA technique, but employs a tag at the 3' end of the first aRNA, improving the yield of full-length transcripts.

Both Eberwine and Serafini presume, and rely on, representative amplification of mRNA populations. The maintenance of this relative representation fundamental to his amplification protocol and Eberwine goes to great length to reassure us that his cDNA population reflects his mRNA population and, because there is no significant difference in the amount of amplification of individual cDNAs into aRNA, his aRNA should also reflect the original mRNA population (see col.5, lines 55-61). In fact, his discussion of internal control molecules to serve as normalization controls is posited to account for any potential differences in amplification

efficiency. Our invention effects the opposite result: instead of maintaining relative differential expression, we eliminate or normalize this differential.

In particular, the present inventors have found that despite the improvements offered by Serafini, they still were unable to detect very weakly expressed transcripts in mRNA populations. The inventors expended substantial efforts to further optimize the amplification protocol in frustrating pursuit of these rare transcripts. Only then, in some desperation did the inventors conceive of combining the linear amplification protocol with a normalization step. Normalization of nucleic acid libraries (such as in Gudkov) is not new - but it has never been applied in such an amplification protocol. Not only was such a combination unprecedented, contrary to the teachings of Eberwine and Serafini, and non-obvious to those of ordinary skill in the art, it was even non-obvious to those of extraordinary skill and familiarity with the art - the coinventors of the cited Serafini et al.

The Examiner is invited to call the undersigned if she would like to amend the claims to clarify the foregoing or seeks further clarification of the claim language.

Applicants hereby petition for any necessary extension of time pursuant to 37 CFR 1.136(a). The Commissioner is hereby authorized to charge any fees or credit any overcharges relating to this communication to our Deposit Account No. 19-0750 (order no. B00-100-1).

Respectfully submitted,
SCIENCE & TECHNOLOGY LAW GROUP

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encl. Expert Declaration under 37CFR1.132, 3 p.